High-selenium yeast supplementation in free-living North American men: No effect on thyroid hormone metabolism or body composition

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Abstract

In a prior study, we observed decreased serum 3,3',5-triiodothyronine (T3), increased serum thyrotropin and increased body weight in five men fed 297 µg/d of selenium (Se) in foods naturally high in Se while confined in a metabolic research unit. In an attempt to replicate and confirm those observations, we conducted a randomized study of high-Se yeast supplements (300 µg/d) or placebo yeast administered to 42 healthy free-living men for 48 weeks. Serum thyroxine, T3 and thyrotropin did not change in supplemented or control subjects. Body weight increased in both groups during the 48-week treatment period and remained elevated for the 48-week follow-up period. Body fat increased by 1.2 kg in both groups. Energy intake and voluntary activity levels were not different between the groups and remained unchanged during the treatment period. Dietary intakes of Se, macronutrients and micronutrients were not different between groups and remained unchanged during the treatment period. These results suggest that our previous observation of a hypothyroidal response to high-Se foods was confounded by some aspect of the particular foods used, or were merely chance observations. Because of the high dose and long administration period, the present study suggests that the effects of Se supplements on thyroid hormone metabolism and energy metabolism in healthy North American men with adequate Se status do not represent a significant risk for unhealthy weight gain.

Keywords: Selenium; Thyroid hormone; Body composition; Body weight

Introduction

Thyroid hormone, 3,3',5-triiodothyronine (T3), affects growth, differentiation and cellular metabolism and plays an essential role during human fetal and childhood development [1]. It binds to two thyroid hormone receptor (Tr) isoforms coded by different genes, which mediate gene expression by binding to thyroid hormone response elements in the promoter regions of target genes. In the absence of thyroid hormone, Trs recruit nuclear co-repressor complexes that lead to histone...
deacetylation of the localized chromatin and lowering of the basal transcription rate of the target genes [2]. In the presence of T₃, Trs recruit co-activator complexes with histone acetyltransferase activity as well as other factors that can associate with RNA polymerase II to initiate transcription of positively regulated target genes. Trs also negatively regulate expression of some target genes, but the mechanisms are not well understood [3].

Iodine is an essential component of thyroid hormone, and iodine deficiency causes brain damage and impaired motor-mental development in the fetus, the neonate and in the child. Populations with severe iodine deficiency suffer from endemic goiter and cretinism, endemic mental retardation, decreased fertility rate, increased perinatal death, and infant mortality [4]. In adults, thyroid hormone regulates thermogenesis [5] and energy metabolism [6], partly by its action on mitochondrial processes [7] and partly through a non-genomic effect on HIF-1α transcription factor, which regulates glucose transport and glycolytic enzymes [8]. Recent DNA microarray studies have revealed new targets of thyroid hormone in liver, a major site of regulation of energy metabolism, including CYP17α (cholesterol synthesis), cysteine dioxygenase (bile acid conjugation), and hydroxymethylglutaryl coenzyme A (HMG-CoA) synthase (ketogenesis) [9]. In addition, gene expression profiling experiments suggest that thyroid hormone modulates numerous cell signaling pathways in both positive and negative directions [10]. Co-deficiency of iodine and Se is thought to underlie the etiology of the severe osteoarthropathy known as Kashin–Beck disease [11] and endemic myxedematous cretinism [12].

The essential trace element selenium (Se) is required for synthesis of selenocysteine at the active sites of selenoenzymes. Although best known as the catalytic moiety in antioxidant enzymes such as glutathione peroxidase (GPX) and thioredoxin reductase, selenocysteine is also essential for the activities of the three iodothyronine deiodinases that control thyroid hormone metabolism. Type I deiodinase (D1) catalyzes the removal of the 5′ (outer ring) iodine from thyroxine (T₄), 3,3′,5′-triiodothyronine (rT₃), and other iodothyronines or the removal of the inner ring iodine from their sulfoconjugates, and has traditionally been viewed as responsible for producing most of the circulating T₃ [13]. D1’s role in energy metabolism is evident because fasting decreases and carbohydrate feeding rapidly increases liver D1 activity via thyrotropin [14]. D1 also receives a priority supply of Se when supply is limited, at least compared to GPX [15]. In fact, there is little evidence to suggest that Se supply limits the activity of any of the deiodinases. Except in hyperthyroidism, D1’s main physiological role seems to be in tissue-specific patterns of deiodination [16] rather than supplying circulating T₃ [17].

Type II deiodinase (D2) is the other outer ring deiodinase that produces T₃ from T₄, and has been considered to act primarily intracellularly in tissues such as brain. Feedback regulation of pituitary thyrotropin excretion depends on D2 to convert T₄ from the circulation into T₃ inside the pituitary cells that can bind to the Tr and thereby regulate thyrotropin synthesis. The identification of membrane transporters specific for T₄ and T₃ in the central nervous system suggests D2 may act in a paracrine fashion, producing T₃ in glial cells that acts on nearby neurons expressing Trs [18]. Insights gained from experiments with recently available mouse deiodinase knockout models suggest that D2 is actually the primary source of circulating T₃, except in hyperthyroidism [16]. The physiological importance of D2 continues to grow, with roles recognized in adaptive thermogenesis [19], G-protein coupled bile acid receptor 1-mediated signaling [20], and Hedgehog signaling in chondrocyte differentiation [21].

Type III deiodinase (D3) removes the 5′ (inner ring) iodine from T₄ or T₃, which abolishes binding to Tr, inactivating the prohormone and active hormone, respectively. D3 seems to act mostly during development, where its expression is regulated in a tissue- and time-specific manner [17].

Because of its reliance on hydrogen peroxide for iodination of thyroglobulin and crosslinking of iodoxyroside residues into iodothyronines, the thyroid gland has an extraordinary need for protection from oxidative damage. H₂O₂ is produced by a thyroid-specific NADPH oxidase located at the apical membrane. The H₂O₂ thus produced diffuses to the luminal space where it reacts with extracellular thyroglobulin and iodide in a reaction catalyzed by thyroperoxidase. Thyroidal H₂O₂ production and thyroperoxidase activity are under nutritional and hormonal control [22]. Thyroid has the highest Se concentration of any tissue in the human body [23] and retains Se tenaciously during nutritional deficiency, suggesting Se has an important role in thyroid physiology. Several selenoproteins are expressed in thyroid, including GPX1, GPX3, D1, D2, thioredoxin reductase, Sep15 and selenoprotein P [17]. GPXs are important antioxidant enzymes that reduce H₂O₂ at the expense of glutathione. GPX3, the extracellular form of the enzyme, is expressed at the apical plasma membrane of thyrocytes where it is believed to be secreted into the follicular lumen and regulate extracellular H₂O₂ levels [23]. Because of its high concentration in the lumen, H₂O₂ can easily diffuse across the apical membrane into the thyrocyte, but cytosolic GPX1 provides an effective defense against intracellular damage [24]. Thioredoxin reductase regulates NADPH-dependent thiol-disulfide exchange reactions crucial to control of the intracellular redox environment and repair of oxidative damage [25]. The specific biochemical functions of the other selenoproteins expressed in thyroid are not known. However, all selenoenzymes so far characterized are redox
enzymes and all Sec-containing proteins have at least some antioxidant properties.

Se supplementation improves weight gain in calves [26] and yearling cattle [27] on Se-deficient pastures and since 1979 has been added to feedlot rations to improve feed efficiency and reproduction [28]. Low serum Se was associated with obesity in women participants in the SU.VI.M.A.X study [29] and Se intake was reported to increase with energy expenditure in French athletes [30]. When we fed high-Se rice and high-Se beef to five men in a metabolic research unit, serum T₃ decreased and body weight increased [31]. The present study was conducted to test the effect of an elevated Se intake, provided as high-Se yeast supplements, on thyroid hormone metabolism and body composition in a larger group of healthy men living in their normal circumstances and consuming self-selected diets.

Subjects and methods

Subjects

The present study was part of a larger investigation into the health effects of long-term, high-level Se supplementation in healthy men. Fifty-four healthy, non-smoking men, aged 18–45 years were randomized to treatment. Only the results pertaining to thyroid hormones, energy metabolism and body composition are presented herein. Other aspects of the main study are reported elsewhere. Potential volunteers were given a physical examination by a nurse practitioner and determined to be in good health. Inclusion criteria were: self-reported absence of disease (hypertension, diabetes, sexually transmitted disease, cancer, etc.); and, clinically normal blood count, blood chemistries and thyrotropin. Exclusion criteria were: tobacco smoking; positive blood test for HIV, hepatitis B, syphilis, or positive urine tests for drugs of abuse (barbituates, benzodiazepines, cocaine metabolites, opiates, amphetamines, and cannabinoids); use of Se shampoos, Se supplements providing more than 50μg/d, thyroid medications, weight loss drugs, or anabolic steroids; more than a 10 lb weight change within the last 6 months; and, exercise or physical training in excess of three 1-h sessions per week. Subjects were paid for their participation. The study protocol was reviewed and approved by the Institutional Review Board of the University of California at Davis School of Medicine, and informed consent was obtained in writing from all subjects.

Experimental protocol

Potential subjects meeting the recruitment criteria were enrolled into a run-in period lasting 3–6 weeks, during which baseline measurements were obtained and compliance was assessed. Non-compliant subjects were dismissed before randomization and are not reported further. Two subjects at a time were randomized to treatment from July 2000 to November 2002, with one subject from each pair randomly assigned to each treatment group by coin flip. Fifty-four men satisfactorily completed the run-in period and were randomized to receive placebo yeast tablets or high-Se yeast tablets for 48 weeks. Neither the subjects nor the study staff were aware of subjects’ treatment assignments. Subjects took their first tablet the same day that all baseline measurements were completed. Subjects visited the Center at least once every 6 weeks during the 48-week supplementation period, and then returned again at 72 and 96 weeks. Visits were scheduled relative to each subject’s first day of supplementation. When visits were missed, the next visit was scheduled based on the first day of supplementation to restore the original schedule. Unused tablets were counted at each visit and were collected at the end of the treatment period to measure compliance. Subjects consumed 93±5.3% of the pills assigned. Forty-two subjects completed the 48-week supplementation period. Samples of blood were obtained twice during the run-in period and then every 6–12 weeks during the 48-week supplementation period, and again at 72 and 96 weeks.

Se supplements

Supplements were provided as high-Se Baker’s yeast (Saccharomyces cerevisiae, strain PN0056) grown aerobically using a Pharmacopoeia-controlled growth medium containing sodium selenite (SelenoPrecise™, 300μg Se per tablet, 3.81μmol Se, Pharma Nord, Denmark). Placebo tablets were compounded identically, except using the same yeast grown without added Se (≤1.3μg Se per tablet, 16.5nmol Se). Tablets contained 0.5 g of spray-dried yeast in an inert binder and were coated with titanium dioxide for an identical appearance, smell and taste. Tablets were provided in 28-tablet bubble packs.

Body composition measurements

Subjects wore standard hospital scrubs for all measurements. Body composition was determined twice during the run-in period and then at 24, 48, 72 and 96 weeks by three independent and complementary methods: (a) Total body electrical conductivity (TOBEC) using the HA-2 body composition analyzer (EM-SCAN, Springfield, IL) was used to estimate fat-free mass, using a prediction equation specific for healthy men [32]. Body fat mass was determined by subtracting fat-free mass from body weight. (b) Dual-energy X-ray
absorptiometry (DEXA) using the lunar prodigy bone densitometer (Encore version 2.26, GE Healthcare/Lunar, Madison, WI) directly estimated percentage body fat and fat-free mass. (c) Bioimpedance spectroscopy (BIS) with a Hydra ECF/ICF Bio-Impedance Spectrum Analyzer (Model 4200, software version 1.00d; Xiton Technologies, San Diego, CA) was used to estimate the volume of extracellular water and intracellular water [33], from which fat-free mass was calculated. Body fat mass was determined by subtracting fat-free mass from body weight.

**Dietary intake assessment**

Energy and nutrient intakes were estimated from 3-d diet records. Subjects were trained by a registered dietitian how to estimate the amount and type of foods eaten. Before the first set of 3-d diet records was collected, subjects maintained 2-d diet records that were reviewed by the dietitian for accuracy and discussed with each subject as part of their training. These records were not used in the final analysis. Twice during the run-in period and then at 24, 48, 72 and 96 weeks, subjects kept a written record of all foods eaten for a 3-d period, always including at least 1 weekday (Monday–Thursday) and at least 1 weekend day (Friday–Sunday). Every diet record was reviewed by a registered dietitian in an interview with the subject for completeness and to resolve any uncertainties regarding food identifications or quantities. Records were analyzed for nutrient contents with the Minnesota Nutrition Data System 5.0 (Nutrition Coordinating Center, Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN), using food composition data derived primarily from the US Department of Agriculture’s National Nutrient Database for Standard Reference [34] to calculate daily energy intake and daily intakes of macronutrients, vitamins, minerals and trace elements, including Se.

**Physical activity measurements**

During the same 3-d periods that food records were kept, subjects wore a heart-rate monitor (Polar Vantage NV™, Polar Electro Inc., Port Washington, NY) except when asleep and carried a handheld computer that automatically prompted them every 60 min to record all physical activities in 10 min increments [35], based on a simplified version of the Bouchard 3-d physical activity record [36]. Subjects chose from a list of seven categories, with each category representing a different metabolic equivalent (MET) (activity/energy expenditure) level. Then they selected the number of minutes (in 10-min increments) that they spent in each category that they chose until all categories added to 60 minutes. At the end of each 3-d monitoring period, the digital activity logs were reviewed for completeness and accuracy by a physiologist. Records for days that were missing valid entries for 25% or more of the waking hours were rejected, and when feasible, another day of activity was recorded. Digital activity log records that were incomplete and could not be re-monitored were recorded as missing values. Each activity number assumed a pre-determined energy expenditure based on METs of the constituent activities, and the energy expended was calculated as time spent in that activity times the energy expenditure rate, adjusted for body weight [37]. The estimated energy expended in each activity was summed to obtain a daily value, and the 3 daily values were averaged to estimate the energy expended per day in voluntary activities at each point in the study.

**Laboratory measurements**

Blood samples were collected in the mornings after an overnight fast. Blood samples for clinical chemistries were clotted, and the serum was separated by centrifugation and refrigerated until analyzed for sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, glucose, calcium, protein, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerols each night at a certified reference laboratory (University of California at Davis Medical Center Pathology Laboratory, Sacramento, CA). Commercially available radioimmunoassay kits were used to measure total and free T₄, total and free T₃, and thyrotropin in serum (Diagnostic Products Corporation, Los Angeles, CA). Triacylglycerols [38], free glycerol [39], beta-hydroxybutyrate [40] and non-esterified fatty acids [41] were measured on a Hitachi 902 centrifugal analyzer (Roche Diagnostics, Indianapolis, IN). Se concentrations were measured by HPLC of the fluorescent derivative formed from reaction with diaminonaphthalene after digestion in a (5:2, v/v) nitric– perchloric acid mixture [42]. Nitrogen was measured in acidified urine using a LECO FP-428 Nitrogen Analyzer (LECO Corp., St. Joseph, MI).

**Statistical analysis**

The Box–Cox approach was used to estimate a power transformation that would stabilize the variances among groups and times for those variables with heterogeneity of variance [43]. In some cases a power transformation was unsuccessful in stabilizing the variances so the rank transformation was used. The SAS (version 9.1.3, SAS Institute, Cary, NC) proc mixed was used to fit a repeated measures model [44]. Group, time and the
interaction, and for dietary intake variables season of year and daily maximum temperature as covariates, were the fixed effects. Subject within groups was the random effect. A first-order autoregressive covariance structure was used to account for the dependencies among the repeated measures. Single degree of freedom contrasts were used to compare the baseline averages with the treatment and follow-up measurements separately for the two groups, and the Bonferroni adjustment for multiplicity was applied \[45\]. Adjusted probabilities less than 0.05 were considered significant.

## Results

### Dietary intake

The daily intake of dietary components is shown in Table 1. The intakes of macronutrients, micronutrients, and Se (135 ± 75 μg/d) did not change during the 48-week treatment period. Average energy intake did not differ by season, although water intake increased slightly with the daily maximum temperature (data not shown). The dietary intake of fructose changed during the study and became significantly different between groups by 96 weeks.

### Body composition

Body weight increased by approximately 1.5 kg in both groups during the 48-week treatment period and remained elevated until 96 weeks (Fig. 1). Body weight changes were not significantly related to dietary intake of energy, macronutrients, or micronutrients. Because DEXA is the least variable body composition method used in this study, only DEXA estimates of lean mass and fat mass are shown in Table 2. Body fat mass increased in both groups by 1.2 kg, accounting for most of the increase in body weight. Body fat mass estimated by TOBEC and BIS showed the same trend (data not shown). The distribution of body water between intracellular and extracellular pools did not change during the study and did not correlate with body weight (data not shown).

### Voluntary activity

Subjects’ self-reported activity records and their 24 h heart monitoring data were used to check for

### Table 1. Composition of subjects’ self-selected diets

<table>
<thead>
<tr>
<th>Diet component, intake/d</th>
<th>Placebo yeast (mean ± SD, N = 20)</th>
<th>High-Se yeast (mean ± SD, N = 22)</th>
<th>Se effecta (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baselineb</td>
<td>48 wecks²</td>
<td>96 weeksd</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>74 ± 23</td>
<td>84 ± 29</td>
<td>90 ± 38</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>319 ± 79</td>
<td>299 ± 88</td>
<td>318 ± 100</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>93 ± 28</td>
<td>95 ± 33</td>
<td>93 ± 39</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>35 ± 23</td>
<td>38 ± 21</td>
<td>42 ± 25</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>36 ± 19</td>
<td>37 ± 16.4</td>
<td>44 ± 31</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>54 ± 28</td>
<td>55 ± 32</td>
<td>52 ± 30</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>144 ± 56</td>
<td>126 ± 65</td>
<td>129 ± 55</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>954 ± 357</td>
<td>960 ± 339</td>
<td>1077 ± 711</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1388 ± 321</td>
<td>1450 ± 388</td>
<td>1463 ± 568</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>353 ± 134</td>
<td>328 ± 126</td>
<td>352 ± 166</td>
</tr>
</tbody>
</table>

\[a\] Repeated measures analysis of variance: Se main effect or Se × time interaction.  
\[b\] Values at the end of the run-in period before starting supplementation. 
\[²\] Values at the end of the 48-week supplementation period. 
\[d\] Values 48 weeks after the end of supplementation. 
\[e\] Treatment groups significantly different at 96 weeks only, NS during supplementation period.
changes in voluntary activity that could affect energy balance. These measures did not change during the study (Table 3), and did not differ by season or ambient air temperature.

### Energy metabolites

Serum levels of thyroid hormone, glucose, cholesterol, insulin, triacylglycerols (Table 4), free glycerol, non-esterified fatty acids, and beta-hydroxybutyrate (data not shown) did not change during the study. Serum urea nitrogen levels dropped initially in the high-Se group and increased in the placebo group, such that the groups became indistinguishable from the placebo group by the end of the 48-week supplementation period. Se has been previously associated with lower serum urea nitrogen levels in animals and humans, but the relationship has not been further explored. Diphenyldiselenide administered to cadmium-intoxicated mice lowers serum urea nitrogen [46], but is not nutritionally available [47]. Sodium selenite (a highly bioavailable form of Se) lowered blood urea nitrogen in aluminum-intoxicated rats [48] and cadmium-intoxicated mice [46]. Se as sodium selenite, selenomethionine or high-Se liver powder decreased plasma urea nitrogen levels in rats treated with mercury [49]. Plasma Se concentration in renal failure patients was inversely correlated with blood urea nitrogen [50]. The present study appears to be the first report of an association between Se

### Discussion

A novel observation in the present study was the apparent disturbance of nitrogen metabolism in subjects taking the high-Se supplements. Both serum urea nitrogen and urinary nitrogen excretion decreased within 12 weeks of starting the high-Se tablets, but became indistinguishable from the placebo group by the end of the 48-week supplementation period. Se has been previously associated with lower serum urea nitrogen levels in animals and humans, but the relationship has not been further explored. Diphenyldiselenide administered to cadmium-intoxicated mice lowers serum urea nitrogen [46], but is not nutritionally available [47]. Sodium selenite (a highly bioavailable form of Se) lowered blood urea nitrogen in aluminum-intoxicated rats [48] and cadmium-intoxicated mice [46]. Se as sodium selenite, selenomethionine or high-Se liver powder decreased plasma urea nitrogen levels in rats treated with mercury [49]. Plasma Se concentration in renal failure patients was inversely correlated with blood urea nitrogen [50]. The present study appears to be the first report of an association between Se

### Table 2. Body composition

<table>
<thead>
<tr>
<th>Body compartment</th>
<th>Placebo yeast (mean ± SD, N = 20)</th>
<th>High-Se yeast (mean ± SD, N = 22)</th>
<th>Se effect&lt;sup&gt;a&lt;/sup&gt; (p)</th>
<th>Time effect (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48 weeks&lt;sup&gt;c&lt;/sup&gt;</td>
<td>96 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77.4 ± 11.9</td>
<td>76.3 ± 9.9</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (DEXA) (kg)</td>
<td>16.3 ± 6.3</td>
<td>15.7 ± 7.0</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean mass (DEXA) (kg)</td>
<td>57.9 ± 9.0</td>
<td>57.7 ± 6.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total body water (kg)</td>
<td>45.8 ± 8.9</td>
<td>44.9 ± 4.9</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Repeated measures analysis of variance: Se main effect or Se × time interaction.

<sup>b</sup>Values at the end of the run-in period before starting supplementation.

<sup>c</sup>Values at the end of the 48-week supplementation period.

<sup>d</sup>Values 48 weeks after the end of supplementation.

### Table 3. Energy balance, energy intake, voluntary activity and heart rate were recorded over the same 3-d periods

<table>
<thead>
<tr>
<th></th>
<th>Placebo yeast (mean ± SD, N = 20)</th>
<th>High-Se yeast (mean ± SD, N = 22)</th>
<th>Se effect&lt;sup&gt;a&lt;/sup&gt; (p)</th>
</tr>
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<tr>
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<td>48 weeks&lt;sup&gt;c&lt;/sup&gt;</td>
<td>96 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Energy intake (MJ/d)</td>
<td>9.62 ± 1.83</td>
<td>10.15 ± 2.31</td>
<td>NS</td>
</tr>
<tr>
<td>Energy expenditure (MJ/d)</td>
<td>11.76 ± 2.52</td>
<td>12.41 ± 2.87</td>
<td>NS</td>
</tr>
<tr>
<td>Average heart rate (beats/min)</td>
<td>76.6 ± 6.9</td>
<td>83.5 ± 6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary creatinine excretion&lt;sup&gt;e&lt;/sup&gt; (g/d)</td>
<td>1.38 ± 0.63</td>
<td>1.49 ± 0.65</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary nitrogen excretion&lt;sup&gt;e&lt;/sup&gt; (mol/d)</td>
<td>1.03 ± 0.29</td>
<td>0.91 ± 0.36</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Repeated measures analysis of variance: Se main effect or Se × time interaction.

<sup>b</sup>Values during the 3-6-week baseline period before starting supplementation.

<sup>c</sup>Values at the end of the 48-week supplementation period.

<sup>d</sup>Values 48 weeks after the end of supplementation.

<sup>e</sup>Urine was collected 2–10 d after diet and activity measurements were made.
urea nitrogen and nitrogen excretion (could be argued that the apparent effects of Se on serum testing corrections across all the variables analyzed, it section. Because we did not apply multiple hypothesis supplementation and decreased urinary nitrogen excretion. Because we did not apply multiple hypothesis testing corrections across all the variables analyzed, it could be argued that the apparent effects of Se on serum urea nitrogen and nitrogen excretion (p = 0.023 and 0.049, respectively) were chance events to be expected in a large set of measurements. However, the random coincidence of parallel decreases in these complementary parameters of whole-body nitrogen over the same time frame is improbable (p ≤ 0.001), suggesting there was a real change in nitrogen metabolism.

The dietary assessment methods we used were not sensitive enough to detect small changes in protein or carbohydrate intake that might underlie the observed changes in nitrogen metabolism [51]; however, there is no reason to expect that Se supplementation would affect protein intake. On the other hand, there was a statistically significant change in fructose intake during the study, with a 9% increase in the placebo group and a 25% decrease in the Se-supplemented group (Table 1). Acute fructose feeding causes increased urinary excretion of uric acid in children and animals [52], an effect attributed to fructose-stimulated increases in de novo purine biosynthesis and subsequent degradation into uric acid [53]. However, feeding 66–90 g/d of fructose to 11 healthy adult subjects for 2 weeks did not lead to any detectable changes in urinary urate [54], so it is difficult to imagine that a change in fructose intake of

### Table 4. Thyroid hormone and blood metabolite status

<table>
<thead>
<tr>
<th></th>
<th>Placebo yeast (mean±SD, N = 20)</th>
<th>High-Se yeast (mean±SD, N = 22)</th>
<th>Se effect (p)</th>
</tr>
</thead>
<tbody>
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<td>Baseline&lt;sup&gt;b&lt;/sup&gt; 48 weeks&lt;sup&gt;c&lt;/sup&gt; 96 weeks&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3,3′,5-Triiodothyronine (T₃) (nmol/L)</td>
<td>1.90±0.38 1.77±0.28 1.94±0.35</td>
<td>2.17±0.43 1.98±0.35 2.12±0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Free T₃ (pmol/L)</td>
<td>41±11 39±7.5 41±7.7</td>
<td>45±7.5 45±17 49±24</td>
<td>NS</td>
</tr>
<tr>
<td>Thyroxine (T₄) (nmol/L)</td>
<td>91±17 92±18 96±20</td>
<td>94±17 92±22 93±25</td>
<td>NS</td>
</tr>
<tr>
<td>Free T₄ (nmol/L)</td>
<td>18±2.7 18±3.0 18±2.8</td>
<td>18±2.7 18±2.2 19±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Thyrotropin (mU/L)</td>
<td>2.30±1.31 2.16±1.11 2.11±1.10</td>
<td>2.10±0.85 1.99±0.87 2.30±1.05</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.21±0.56 1.13±0.52 1.28±0.80</td>
<td>1.12±0.56 1.06±0.50 1.02±0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.60±0.98 4.73±0.91 4.89±0.93</td>
<td>4.53±0.91 4.42±1.27 4.40±0.93</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.24±0.44 1.19±0.39 1.22±0.31</td>
<td>1.14±0.23 1.03±0.18 1.06±0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.83±0.59 4.80±0.46 5.04±0.53</td>
<td>4.97±0.54 4.90±0.56 4.92±0.44</td>
<td>NS</td>
</tr>
<tr>
<td>Urea nitrogen (mmol/L)</td>
<td>5.00±1.43 4.86±1.46 4.61±1.21</td>
<td>4.93±1.61 4.39±1.21 3.93±0.82</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>70.7±3.2 69.8±4.2 68.7±3.4</td>
<td>72.2±4.4 70.4±3.9 69.9±4.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Repeated measures analysis of variance: Se main effect or Se × time interaction.
<sup>b</sup>Values at the end of the run-in period before starting supplementation.
<sup>c</sup>Values at the end of the 48-week supplementation period.
<sup>d</sup>Values 48 weeks after the end of supplementation.

![Fig. 2. Urinary nitrogen excretion. Points represent the mean daily Se excretion in urine (± SEM) for subjects treated with placebo yeast (■) or high-Se yeast (●). Asterisks designate the time points at which the group means were significantly different.](image1.png)

![Fig. 3. Blood urea nitrogen. Points represent the mean urea nitrogen concentration in serum (± SEM) for subjects treated with placebo yeast (■) or high-Se yeast (●). Asterisks designate the time points at which the group means were significantly different.](image2.png)
only 3–9 g/d could account for the changes in nitrogen metabolism in the present study.

Experimental hyperthyroidism in rats decreased urinary nitrogen by 30% and increased fecal nitrogen excretion by 80% [55]. It would require only a relatively small change in fecal nitrogen excretion to explain the observed changes in blood urea nitrogen and urinary nitrogen excretion, making this an attractive possibility. Unfortunately, we did not measure fecal nitrogen, so any changes in fecal nitrogen excretion would not have been detected. There is some evidence the subjects in the high-Se group may have been in a relatively hyperthyroid metabolic state compared to the subjects in the placebo group. This seems plausible because the high-Se group tended to have higher heart rates and higher serum free and total T₃, as well as lower thyrotropin compared to the placebo subjects. Because most of the above-mentioned differences in thyroid parameters were present at the beginning of the study and were not significantly different between groups, we do not attribute them to Se supplementation.

Increasing Se intake with high-Se yeast supplements did not change circulating levels of thyroid hormones. Total and free T₄ and T₃ and thyrotropin did not change significantly in either group and were not different between groups at any time in the study. Based on the observed within-subject variability in these response variables we calculated an upper limit to the size of any undetected effects of dietary Se intake on T₄ (8.6 nmol/L), fT₄ (1.2 nmol/L), T₃ (0.14 nmol/L), fT₃ (9.4 pmol/L) and thyrotropin (0.51 mU/L). Therefore, the changes in T₃ and thyrotropin that we observed in our prior study with high-Se foods have not been reproduced by Se supplements in the present study. We also failed to observe an effect of Se on body weight, body fat, or serum triglyceride – the physiological signs of altered thyroid metabolism that we found in our previous study. Thus, the present study provides no support for the notion that use of high-Se yeast supplements alters thyroid metabolism or induces a sub-clinical hypothyroid state in healthy men.

Even though the Se in our supplements did not alter energy metabolism in the subjects, both groups nevertheless gained weight at an increased rate during the 48 weeks they consumed the yeast tablets. Subjects in both groups steadily gained an average of approximately 1.5 kg throughout the 48 weeks they consumed the yeast tablets. Subjects maintained this weight gain and then did not gain or lose significant weight during the 48-week follow-up period. The weight increase during the treatment period exceeded the typical age-related weight increase in healthy men of 0.3–0.8 kg/year [56–58]. The observed weight increases are not consistent with the apparent negative energy balance indicated by the energy intake and energy expended in voluntary activities (Table 3). Both groups reported 1.2–2.8 MJ/d more energy expended in voluntary activities than they reported eaten. There is a well-documented bias towards under-estimation in self-reported energy intakes [59]. Because our free-living subjects were eating self-selected diets, we were not able to control or correct for this bias. Activity records such as Bouchard diaries [36], which were the model for the handheld computer activity records used in the present study, over-estimate the energy expended in voluntary activities compared to accelerometers [60]. Thus, both energy estimation methods were subject to systematic biases that could have contributed to the apparent negative energy balance. In any event, there is no doubt the subjects were in positive energy balance because the subjects gained an average of 1.2 kg fat during the supplementation period (p = 0.004, Table 2).

The fact that the weight increase occurred throughout the supplementation period and remained about constant during the follow-up period suggests that it was related to some aspect of being in the first 48-week treatment period of the study that was common to both groups. The tablets contained 0.5 g dried Saccharomyces cerevisiae, which can increase body weight in dairy calves [61] and broiler chicks [62], and Brewer’s yeast is prescribed as an appetite stimulant in herbal medicine [63]. However, it seems unlikely that 0.5 g/d of yeast could have had such a large effect. It seems more probable that the more frequent clinic visits and intense medical monitoring during the first 48 weeks affected subjects behaviors, and that led to the weight change. Such an effect would be attenuated during the second 48-week follow-up period when clinic visits were much less frequent and when body weights were also more stable. The minimum increase in energy intake we could have detected was 0.99 MJ/d, greater than the 0.12 MJ/d increase in energy intake needed to accumulate 1.2 kg of body fat over 48 weeks. Thus, we cannot rule out the possibility that factors related to study participation might have stimulated appetite, leading to the weight increase in both groups.

Decreased voluntary activity could have contributed to the observed weight gain. Subjects’ activity levels might have been influenced by their mood, which Se supplementation was reported to improve in British volunteers [64]. We assessed subjects’ moods with the profile of mood states (POMS) [65], the same instrument used in the cited study, but we detected no change in mood over time or as a function of Se supplementation (data not shown). Recently a much larger Se supplementation trial in the United Kingdom failed to find any effect of supplementation with 300 μg/d Se on mood assessed with the POMS questionnaire, even in a population with low pre-supplementation Se status [66]. The significance of Se’s effect on mood is still an open question. In any case, it seems unlikely to have played
any role in the present study because we observed no differences in mood or activity associated with Se supplementation. The weight increase could also be related to a slight, but statistically non-significant, 8% decrease in serum T3 during the supplementation period (see Table 4). However, such a shift in serum T3 could be a cause as well as a consequence of altered energy metabolism, because T3 is known to respond to [67], as well as control [68,69], changes in energy metabolism. In any event, the weight gain occurred equally in both treatment groups and was not related to Se supplementation. Although our data show no differences in voluntary activity, we cannot rule it out as a potential contributor to the weight gain because our methods were only able to detect a change of 0.64 MJ/d in activity, whereas a decrease of only approximately 0.12 MJ/d in activity can accumulate excess energy intake equivalent to the average 1.2 kg of body fat gained over 48 weeks. Consequently, we cannot rule out the possibility that factors other than Se related to participation in the treatment phase of the study might have decreased activity, leading to the weight increase in both groups.

The conclusions of the present study are strikingly different from the results of our previous study of men confined in a metabolic research unit for 120 days and fed rice and beef naturally high or low in Se. Although our prior study was shorter in duration and included only 11 subjects, the spectrum of physiological changes we observed in subjects eating the high-Se foods was wholly consistent with a perturbation of thyroid hormone metabolism, i.e. – decreased serum T3, increased serum thyrotropin, and increased body weight. Furthermore, the subjects eating the low-Se foods experienced opposite and complementary changes in energy metabolism: increased serum T3, decreased body weight, loss of body fat and increased serum triacylglycerols. Clearly, thyroid hormone metabolism in our prior study was disturbed by eating high-Se beef from South Dakota, low-Se beef from New Zealand, high-Se rice from Enshi county in China and low-Se rice from Mianning county in China. How then, can we reconcile our prior study with the complete lack of effects of Se supplementation on energy metabolism in the present study?

One possibility is that our earlier observations represented a transient effect of Se that was only apparent in the 89 days the subjects were fed the high- and low-Se foods, and which was not discernable over the 48-week intervention in the present study. That this might be the case is suggested by the greater rate at which subjects in the high-Se group appeared to gain weight at the beginning of the present study (Fig. 1). This difference, although not statistically significant, was maximal at 6 weeks and disappeared by 18 weeks, suggesting a transient change in energy metabolism occurred in the present study. The effect of dietary Se on serum T3 also appeared transient in our prior study and was attenuated 42% within 68 days [31]. On the other hand, serum T3 did not change during the first 18 weeks of the present study, and the Se-supplemented group tended to have higher, not lower, serum T3 concentrations during that period (data not shown).

An alternative explanation for the different outcomes in the present study and our prior study is that dietary components other than Se influenced thyroid hormone metabolism in the prior study. The diet fed to the subjects in the prior study was low in calcium (57% of RDA) and magnesium (46% of RDA). Calcium intake has been shown to modulate thyroid hormone metabolism in rats [70,71]. Similarly, magnesium intake affects thyroid hormones in chicks [72] and rats [73]. However, it is difficult to imagine how these deficiencies, which were present in the diets of both groups, could have led to a difference in thyroid hormone metabolism between groups. On the other hand, the differences in thyroid hormones observed in our prior study might have been due to an unmeasured dietary component. In our prior study we did not analyze the diets for iodine, which is very low in New Zealand soils [74], but is abundant in South Dakota, which once lay under an inland sea [75]. Thus it is possible that the high-Se beef in our prior study contained more iodine as well. High iodine intake can transiently depress thyroid function [76], which might have contributed to the transient hypothyroidal response to the high-Se foods. Goitrogenic substances that interfere with thyroid metabolism may have been different between the high-Se and low-Se foods, but neither rice nor beef has been reported to contain significant quantities of goitrogens. There may have been other differences in the compositions of the high-Se and low-Se foods that affected thyroid hormone metabolism in our prior study. We cannot be certain why the high-Se foods in our prior study affected thyroid hormone metabolism. However, the results of the present study provide strong evidence that increased Se intake from high-Se yeast supplements does not change thyroid hormone or energy metabolism to an appreciable extent in healthy free-living North American men eating self-selected diets. In keeping with this conclusion, weight gain has not been reported in previous trials of high-level Se supplementation using a variety of forms of Se in different populations [66,77–84]. Clinical trials suggest that supplementation with high-Se yeast can prevent cancer [77] and the National Cancer Institute is conducting a large trial in 32,000 men to test Se against prostate cancer [85]. Accordingly, there is no reason to think that use of Se supplements for cancer prevention would increase the risk of unhealthy weight gain.
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